

to be involved in direct Ca^{2+} -binding but may provide intersubunit supporting points to mediate Ca^{2+} -gated RCK gating ring expansion or conformational coupling to channel activation.

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Functional Implications of Alternative Splicing in the Calcium-Activated BK Channel in the Ampulla of Lorenzini of the Skate

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Ca-activated K channels were first described in the skate Ampulla of Lorenzini, which has excitable receptor cells. The electroreceptor epithelium generates action potentials (up to 70 mV) which don't repolarize unless calcium enters across the apical membranes of the receptor cells. We have recently cloned and sequenced the alpha-subunit of the B_K channel of the skate in late-stage embryos and then in adult ampullae. Sequencing of embryos was accomplished using transcriptome m-RNA and then genomic DNA. Millions of short reads were assembled by alignment with an isoform of the human B_K channel. The human isoform had regions of nucleotides that were excluded to get correct alignment with the skate sequences. The skate embryo alpha- B_K gene was used to make PCR primers for amplification of cDNA obtained by reverse transcription of mRNA from about 100 adult ampullae. Amplified cDNA of appropriate length was then cloned into E.Coli with PCR2 vector (Invitrogen). The skate embryos and ampullae have a 1114 (vs. 1118) amino acid polypeptide that begins with MEVP and may be preceded by 33 additional AA's. The ampulla sequence had 100% coverage of the embryo alpha subunit and was 96.7% homologous at the amino acid level. There are 7 alternative splicing sites in the alpha-subunit. Most non-homology of ampulla B_K channels vs. skate embryos was at these sites. Seven separate point mutations were also found. The calcium-activated channel in the ampulla appears to be voltage-insensitive, in contrast to previously sequenced B_K channels. The voltage sensor is normal in ampullae, but changes at other sites could produce voltage-insensitivity.

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Structural and Thermodynamic Characterization of the Gating Pathway in a K^+ Channel

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Structures of inwardly-rectifying (Kir) potassium channels are now available in many different crystallographic states. We now analyse these structures in the context of functional data for mutations at over 180 positions within the Kir1.1 (ROMK) channel. This reveals an extensive network of physically interacting residues which stabilise the pre-open and open-states of the channel, but which breaks down upon channel closure. This approach not only validates a structural gating pathway for the Kir channel, but also provides insight into the structure of the transition state connecting these crystallographic states. This gating network also appears to be an important structural determinant of the thermodynamic stability of these different gating states as well as influencing the impact of mutations on channel function, and suggest that such state-dependent physical connectivity between residues may also be relevant to our understanding of other allosteric proteins.

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HCN C-Terminal Region Speeds Activation Independently of Autoinhibition

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The C-terminal Cyclic Nucleotide Binding (CNB) fold of HCN channels preferentially stabilizes the closed state, and this "autoinhibition" is relieved by cAMP binding or by CNB fold truncation [Wainger et al. (2001) *Nature* 411: 805]. Whereas the autoinhibition model was discovered in excised-patch conditions, we investigated the ability of the C-terminal region to regulate gating thermodynamics and kinetics with two-electrode voltage clamp on *Xenopus* oocytes. We examined wildtype channels (HCN2) with endogenous cAMP bound, C-terminal-truncated channels (HCN2 ΔCNB), and channels with autoinhibition imposed due to a mutation preventing cAMP binding (HCN2 R591E). Hyperpolarized $V_{1/2}$ and faster deactivation $t_{1/2}$ values were observed for HCN2 R591E, verifying its autoinhibition in intact oocytes. Surprisingly, we found conditions where the autoinhibition model is insuffi-

cient to explain the hyperpolarization-dependent activation kinetics. First, channels with autoinhibition relieved by cAMP binding exhibited faster activation (HCN2, 81 ± 17 ms at -150 mV) than channels with autoinhibition wholly removed by CNB fold truncation (HCN2 ΔCNB , 215 ± 110 ms at same voltage). Second, autoinhibited channels opened at a relatively fast rate (HCN2 R591E, 77 ± 16 ms) similar to the autoinhibition-free HCN2. We therefore propose a new "quickenning element" in the C-terminal region which works distinctly from and opposite to autoinhibition in determining activation kinetics. The quickening effect of this element in HCN2 and HCN2 R591E were observed for voltages from -110 mV through -160 mV when compared to HCN2 ΔCNB . At -100 mV, as HCN2 R591E opened slower (385 ± 46 ms) than HCN2 (238 ± 33 ms) as predicted by the autoinhibition model. Therefore in intact oocytes, the autoinhibition and the quickening element mechanisms exert two opposing influences on HCN activation speeds with a balance that changes with voltage.

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Lipid Modulation of a Dual Function TMEM16 Channel/Scramblase

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The TMEM16 family of Ca^{2+} activated ion channels plays key roles in human physiology. Surprisingly, recent evidence suggested that some family members are not (only) ion channels but that some are phospholipid scramblases, proteins that mediate the bi-directional movement of lipids between leaflets of the membrane. For example, TMEM16F has been reported to be an ion channel and/or a lipid scramblase. Recently, our group identified a novel fungal TMEM16 homologue, aTMEM16, which is both a Ca^{2+} -gated ion channel and a Ca^{2+} -dependent phospholipid scramblase. We found that membrane composition is a key regulator of ion channel gating but not of phospholipid scrambling activity, as we were able to suppress channel activity without altering scrambling activity by changing the lipid composition of the membranes. This suggested that aTMEM16 forms separate transmembrane pathways for ions and lipids. Here we investigate the differential modulation by lipids of the ion channel and lipid scrambling functions of aTMEM16. Preliminary experiments suggest that the presence of 10 mole percent or more phosphatidylcholine (PC) or cardiolipin is required for channel activity. In contrast, lipid scrambling occurs with similar properties in all membrane compositions tested. Currently we are systematically investigating the effect of lipid headgroup charge, chain length and stiffness on both functions. Our results suggest that modulation by lipids might be a regulatory mechanism for cells to control both functions, for example by compartmentalizing these proteins in membrane domains with different lipid compositions where only one pathway is active.

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YidC Alters Conductivity and Ion Selectivity of the Bacterial Translocation Channel SecYEG

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During protein transport across the membrane the bacterial translocon SecYEG must maintain the barrier to cations and protons in order to preserve the proton motif force. In vivo and in vitro experiments with SecYEG mutants in the bacterial plasma membrane attributed the preservation of the proton motif force to an intrinsic anion-selectivity of the channel. Since potential residues responsible for selective cation exclusion are not easily recognizable in the SecYEG structure we tested the ion selectivity of (i) the purified wild type SecYEG channel, which was opened by ribosome binding1 and (ii) the purified plugless SecYEG mutant2 after reconstitution into planar bilayers. In both cases, reversal potential measurements under conditions of a transmembrane salt gradient did not reveal physiologically relevant ion selectivity. Upon coreconstitution of SecYEG with YidC we found decreased single ion channel conductivity and increased anion selectivity of SecYEG. Combined, both factors acted to decrease the cation leak through SecYEG by an order of magnitude. Additional experiments on YidC reconstituted into planar bilayers suggest that YidC itself forms a pore which is opened by ribosome binding.

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2. Saparov S., Erlandson K., Cannon K., Schaletzky J., Schulman S., Rapoport T., Pohl P. (2007) *Mol Cell*, 26, 501-509.